

27. The expression cassette of claim 26 wherein the signal sequence is AprE.

28. The expression cassette of claim 26 wherein the signal sequence is the  
5 *B. licheniformis*  $\alpha$ -amylase (AmyL) signal peptide.

29. The expression cassette of claim 25 wherein the signal sequence is for the Twin Arginine Translocation secretory pathway.

10 30. A recombinant protein of interest comprising a protein of interest covalently attached at its C-termini to a tag.

31. The recombinant protein of interest of Claim 29 wherein said tag is at least one amino acid residue wherein said residue is a charged residue.

15 32. The recombinant protein of interest of Claim 30 wherein said charged residue is negatively charged.

33. The recombinant protein of interest of Claim 31 wherein said negatively  
20 charged residue is D.

34. The recombinant protein of interest of Claim 31 wherein said negatively charged residue is E.

25 35. The recombinant protein of interest of Claim 30 wherein said charged residue is positively charged.

36. The recombinant protein of interest of Claim 34 wherein said positively charged residue is K.

37. The recombinant protein of interest of Claim 34 wherein said positively charged residue is N.

38. The recombinant protein of interest of Claim 29 wherein said tag is  
5 selected from the group comprising SsrA<sup>NN</sup> (SEQ ID NO:\_\_\_), SsrA<sup>DD</sup> (SEQ  
ID NO:\_\_\_), SsrA<sup>KK</sup> (SEQ ID NO:\_\_\_), and SsrA<sup>EE</sup> (SEQ ID NO:\_\_\_).

39. A chimeric polypeptide comprising (i) a secretion signal peptide, (ii) a  
heterologous polypeptide and (iii) a tag sequence.

40. The chimeric polypeptide of Claim 39 wherein the secretion signal  
peptide is selected from sec-dependent or tat-dependent secretion signals.

41. The chimeric polypeptide of Claim 40 wherein the secretion signal  
15 peptide is a tat-dependent secretion signal.

42. The chimeric polypeptide of Claim 41 wherein the secretion signal  
peptide is selected from PhoD or LipA derived from *Bacillus*.

43. The chimeric polypeptide of Claim 40 wherein the secretion signal  
20 peptide is a sec-dependent secretion signal.

44. The chimeric polypeptide of Claim 43 wherein the secretion signal  
peptide is selected from AmyL or AprE secretion signal peptides.

45. A nucleic acid molecule comprising a first nucleotide sequence encoding  
a signal sequence operatively linked to a second nucleotide sequence  
encoding a heterologous polypeptide wherein the last two codons of the  
polypeptide have been replaced with codons for a charged amino acid  
30 residue.

46. The nucleic acid molecule of claim 45 wherein the charged amino acid residue is positively charged.

5 47. The nucleic acid molecule of claim 46 wherein the charged amino acid residue is K.

48. The nucleic acid molecule of claim 46 wherein the charged amino acid residue is N.

10 49. The nucleic acid molecule of claim 45 wherein the charged amino acid residue is negatively charged.

50. The nucleic acid molecule of claim 49 wherein the charged amino acid residue is D.

15 51. The nucleic acid molecule of claim 49 wherein the charged amino acid residue is E.

20 52. A nucleic acid molecule comprising a first nucleotide sequence encoding a signal sequence operatively linked to a second nucleotide sequence encoding a heterologous polypeptide and a third nucleotide sequence encoding a tag sequence.